AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims

1. (original) A method for multiplex detection of methylation of target nucleic acids comprising:

(a) providing a first population of target nucleic acids labeled with a purification tag;

(b) cleaving said first population of target nucleic acids with an enzyme, whereby said enzyme discriminately cleaves at methylation target sequences forming a second population of cleaved target sequences;

(c) immobilizing said first and second populations by said purification tag; and

(d) detecting the presence of said first population comprising non-cleaved target nucleic acid whereby the presence of said first population comprising non-cleaved target nucleic acid indicates the presence of methylated target nucleic acids.

2. (original) The method according to claim 1, wherein said purification tag comprises biotin and said first and second labeled target nucleic acids are immobilized with streptavidin.

3. (original) The method according to claim 1, wherein said enzyme is selected from the group consisting of HpaII, MspI and DraI.

4. (original) The method according to claim 1, wherein said detecting comprises:

(e) contacting said immobilized first and second populations with a composition comprising a plurality of target probes forming a plurality of hybridization complexes, said probes comprising:

(i) a first region complementary to a first region of a target nucleic acid; and

(ii) a second region comprising a detection sequence complementary to a potentially methylated nucleotide; and

(f) detecting the presence of said probe as an indication of the presence of methylated target nucleic acid.

5. (original) The method according to claim 4, wherein said probes further comprise at least a first universal priming sequence and f) comprises:

(i) contacting said hybridization complexes with a composition comprising:

a) at least first universal primers;

b) dNTPs; and

c) polymerase,

whereby said probes are amplified forming a plurality of amplicons; and

(ii) detecting said amplicons as an indication of the presence of methylated target nucleic acid.

6. (original) A method of detecting methylation comprising:

(a) contacting a sample of target nucleic acids with bisulfite, whereby non-methylated cytosine is converted to uracil, and methylated cytosine is not converted to uracil;

(b) contacting said treated target nucleic acids with a first probe that hybridizes with a methylated target in said first population of target nucleic acid and a second probe that hybridizes with a non-methylated target in said second population of target nucleic acid, forming first and second hybridization complexes, respectively;

(c) contacting said first and second hybridization complexes with an enzyme that modifies said first and second probes forming first and second modified probes;

(d) detecting said first and second modified probes to determine the presence of methylation in said target nucleic acid.

7. (original) A method according to claim 6, wherein said detecting comprises:

(i) contacting said hybridization complexes with a composition comprising:

a) at least first universal primers;

b) dNTPs; and

c) polymerase,

whereby said probes are amplified forming a plurality of amplicons; and

(ii) detecting said amplicons as an indication of the presence of methylated target nucleic acid.

8. (original) The method according to claim 5 or 7, wherein said probes are amplified by a method selected from the group consisting of oligonucleotide ligation assay (OLA), polymerase chain reaction (PCR) and rolling circle amplification (RCA).

9. (original) The method according to claim 8, wherein said probes are amplified by oligonucleotide ligation assay (OLA).

10. (original) The method according to claim 8, wherein said probes are amplified by polymerase chain reaction (PCR).

11. (original) The method according to claim 8, wherein said probes are amplified by rolling circle amplification (RCA).

12. (original) The method according to claim 5 or 7, wherein said amplicons are detected by hybridizing said amplicons to an array.

13. (original) The method according to claim 12, wherein said array is selected from the group consisting of an ordered array, a liquid array and a random array.

14. (original) The method according to claim 5 or 7, wherein said amplicons are detected by mass spectrometry.

15. (original) The method according to claims 5 or 7, wherein said amplicons are detected by capillary electrophoresis.

16. (new) A method for generating a calibration curve for the quantitative methylation measurement of an unknown sample, comprising

(a) obtaining a virtually unmethylated template population having nucleotide sequences corresponding to the nucleotide sequences of a reference genomic DNA;

(b) obtaining a methylated template population comprising nucleotide sequences corresponding to said nucleotide sequences of said reference genomic DNA; and

(c) separately mixing fixed amounts of said virtually unmethylated template population with fixed amounts of said methylated template population at various distinct ratios to create a series of mixtures that represents a methylation gradient, wherein a calibration curve is generated for quantitative methylation measurements.

17. (new) The method of claim 16, wherein said reference genomic DNA is amplified at least one hundred fold.

18. (new) The method of claim 16, wherein said reference genomic DNA is amplified at least ten thousand fold.

19. (new) The method of claim 16, wherein step (a) comprises amplifying a reference genomic DNA, wherein the ratio of methylated to unmethylated sequences in the amplified product is decreased sufficiently to provide said virtually unmethylated population of nucleic acids.

20. (new) The method of claim 16, wherein said methylated template population is obtained by methylating a portion of said virtually unmethylated population of nucleic acids.

21. (new) The method of claim 16, wherein said methylated template population is obtained by methylating said reference genomic DNA.

22. (new) The method of claim 16, wherein said methylated template population is obtained using an enzyme biological sample, or fraction thereof having DNA methylation activity.

23. (new) A method for measuring the extent of methylation for a sample genomic DNA, comprising

(a) obtaining a virtually unmethylated template population having nucleotide sequences corresponding to the nucleotide sequences of a reference genomic DNA;

(b) obtaining a methylated template population comprising nucleotide sequences corresponding to said nucleotide sequences of said reference genomic DNA;

(c) separately mixing fixed amounts of said virtually unmethylated template population with fixed amounts of said methylated template population at various distinct ratios to create a series of mixtures that represents a methylation gradient, wherein a calibration curve is generated for quantitative methylation measurements;

(d) measuring a methylation signal for a sample genomic DNA; and

(e) comparing the intensity of said methylation signal with said calibration curve, thereby measuring the extent of methylation for said sample genomic DNA.

24. (new) The method of claim 23, wherein said reference genomic DNA is amplified at least one hundred fold.

25. (new) The method of claim 23, wherein said reference genomic DNA is amplified at least ten thousand fold.

26. (new) The method of claim 23, wherein step (a) comprises amplifying a reference genomic DNA, wherein the ratio of methylated to unmethylated sequences in the amplified product is decreased sufficiently to provide said virtually unmethylated population of nucleic acids.

27. (new) The method of claim 23, wherein said methylated template population is obtained by methylating a portion of said virtually unmethylated population of nucleic acids.

28. (new) The method of claim 23, wherein said methylated template population is obtained by methylating said reference genomic DNA.

29. (new) The method of claim 23, wherein said methylated template population is obtained using an enzyme biological sample, or fraction thereof having DNA methylation activity.